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55. Morizo Ishidate,[*1] Shoji Takitani,[*2] and Toyokazu Kishi[*1]: Arylamine N-Glucuronide and its Structure.[*3]

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Recently it was shown by several investigators[1~5] that some aromatic primary amines are metabolized to form labile N-glucuronide conjugated with glucuronic acid and excreted in urine. The formation of such labile N-glucuronide *in vivo* offers an interesting problem on metabolism of drugs and pharmacology.

It is remarkable that aromatic primary amines are generally able to form the corresponding N-glucuronides by a simple admixture with glucuronate or glucuronic acid in an aqueous medium. The present paper describes the synthesis of N-glucuronide of several amines and also the fact that the N-glucuronides obtained have a structure of β-glucopyranoside which possibly remains valid as N-glucuronide *in vivo*.

I. Preparation of Arylamine N-Glucuronide

In 1889, Thierfelder[6] first obtained a condensation product in a small yield by heating a mixture of aniline and potassium glucuronate in 90% ethanol. A series of N-glucuronide was obtained by the present authors in a fairly good yield in a following manner: An acetone-water (1:1) solution of amine (1.5 mol. equiv.) was added to a concentrated solution of glucuronate, the mixture was adjusted to pH 5~6, and after a short heating, left at room temperature shielded from light. The separated product was washed with ethanol and ether, and purified by reprecipitation from the aqueous solution by addition of acetone. N-Glucuronides of aniline,[*4] toluidine,[*4] *p*-chloroaniline, *p*-acetamidoaniline, *p*-dimethylaminoaniline, *p*-aminoazobenzene, and *p*-phenylenediamine were prepared successfully through this procedure.

In the case of *p*-nitroaniline, a weaker base, the corresponding N-glucuronide was not formed by this procedure but the formation of the glucuronide was demonstrated by the evidence on paper chromatogram. When ammonium glucuronate or glucuronic acid was employed instead of sodium salt in the above preparation, different compounds were formed. For example, in the case of aniline and toluidine, the product is composed of 2 moles of the amine and one mole of glucuronate or glucuronid acid. The fact that the compound is nothing but an addition product of one mole of the amine to N-glucuronide was confirmed by its separation into the two components by the application of paper electrophoresis. When ethyl glucuronate was added to ethanol solution with primary aromatic amines, such as aniline or *p*-aminoazobenzene, the corresponding ethyl N-glucosyluronate formed easily.

On the other hand, in the case of secondary aromatic amines, such as methylaniline and ethylaniline, there was observed no reaction with glucuronic acid and glucuronate under both conditions mentioned above.

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*3 A part of this work was published in Pharm. Weekblad, **93**, 216(1958).
*4 The N-glucuronide has been reported, but without data.

1) J. N. Smith, R. T. Williams: Biochem. J., **44**, 242(1949).
2) *Idem.*: *Ibid.*, **44**, 250(1949).
3) E. Boyland, D. Manson: *Ibid.*, **60** (No. 1), ii (1955).
4) E. Boyland, D. Manson, S. F. D. Orr: *Ibid.*, **65**, 417(1957).
5) S. R. M. Bushby, A. J. Woiwod: *Ibid.*, **63**, 406(1956).
6) H. Thierfelder: Z. physiol. Chem., **13**, 275(1889).

An attempt was made to obtain N-glucuronide of secondary amines through the hydrolysis of tri-O-acetyl-N-glucuronate. These compounds were formed as the condensation product of N-methylaniline, N-ethylaniline, or *p*-methylaminoazobenzene with methyl 2,3,4-tri-O-acetyl-(glucopyranosyl bromid)uronate. However, the attempt to obtain the N-glucuronide of secondary amines in a pure state by hydrolysis was not successful because of the instability of these compounds to hydrolysis.

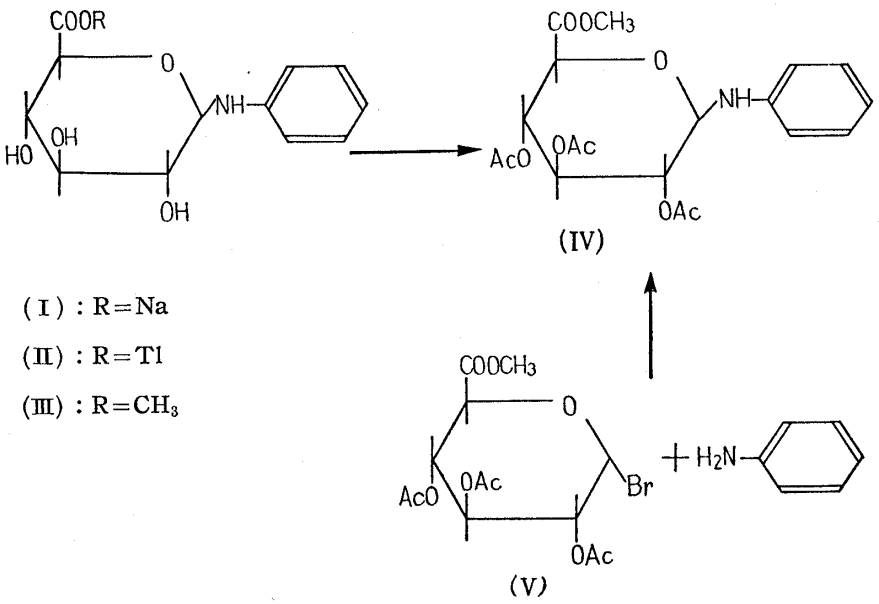
II. Structure of the Glucuronic Acid Conjugate

The glucuronic acid conjugates of aniline,[7] naphthylamine,[8] *p*-aminoazobenzene,[8] and 4,4′-diaminodiphenyl sulfone[5] were detected from urine and have been proved by paper chromatography, and these compounds are identical with the synthesized specimen.

It is natural to suppose that the conjugates formed *in vivo* and *in vitro* would be a compound of the same structures, e.g. having glucopyranoside linkage. However, up to the present, there has been no report that the conjugate really possesses that structure.

In order to solve this problem, the following experiment was carried out. Thallium aniline N-glucosyluronate was first prepared by either reacting aniline with thallium glucuronate or sodium aniline N-glucosyluronate with thallium nitrate. Then this thallium salt was converted to its methyl ester according to the procedure of Micheel and Habendorff,[9] and the product obtained was acetylated with acetic anhydride in pyridine to methyl tri-O-acetylaniline N-glucosyluronate (m.p. 175°). The same compound, on the other hand, was also obtained through the usual procedure of β-glucoside preparation, e.g. by the condensation of methyl (2,3,4-tri-O-acetylglucopyranosyl bromid)uronate with aniline in the presence of silver carbonate.

In all respects, both specimens were proved to be identical and the fact was further confirmed by characteristic infrared absorption band at 1040 cm^{-1}, indicating the presence of a pyranosidic ether linkage.[10]



From these facts, it would be concluded that aniline N-glucuronide and N-glucuronic acid conjugate of aromatic amine both have a structure of N-glucopyranoside, possibly in β-form.

7) M. Ishidate, *et al.* : Pharm. Weekblad, **93**, 216(1958).
8) M. Ishidate, Y. Hashimoto : This Bulletin, **7**, 108(1959).
9) F. Micheel, R. Habendorff : Ber., **90**, 1590(1957).
10) M. Ishidate, T. Nakajima : This Bulletin, **6**, 433(1958).

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Experimental

Sodium Aniline N-Glucosyluronate (I)—A mixture of a solution of aniline (10 g.) in acetone (10 cc.) and an aqueous solution (10 cc.) of sodium glucuronate (2 g.) was left to stand overnight after adding a drop of AcOH. The separated crystalline product was washed with EtOH and Et_2O. Recrystallization by precipitation from an aqueous solution with acetone with cooling gave leaflets, m.p. 198~200°(decomp.); yield, 2.3 g. $[\alpha]_D^{26}$ −94.50° → −35.67°(c=2.38, H_2O). Paper chromatography (descending method) using Toyo Roshi No. 523 and solvent-system of PrOH-*n*-BuOH-0.2*N* NH_4OH (2 : 1 : 1) gave a spot at Rf 0.29, which colors with both Ehrlich and aniline hydrogen phthalate reagents. *Anal.* Calcd. for $C_{12}H_{14}O_6NNa$: C, 49.47; H, 4.87; N, 4.82. Found : C, 49.34; H, 4.57; N, 4.99.

Addition Product of Ammonium Aniline N-Glucosyluronate and Aniline—Prepared from ammonium glucuronate (1 g.) and aniline (5 cc.) by the same procedure; m.p. 130°(decomp.). Yield, 1.1 g. $[\alpha]_D^{24}$ −54.59° → −14.84°(c=2.29, H_2O). *Anal.* Calcd. for $C_{18}H_{25}O_6N_3 \cdot H_2O$: C, 54.40; H, 6.85; N, 10.57. Found : C, 54.40; H, 6.90; N, 10.68.

Sodium *p*-Toluidine N-Glucosyluronate—Prepared by the same procedure. Leaflets recrystallized from water and MeOH, m.p. 175~179°(decomp.). $[\alpha]_D^{24}$ −74.5° → −17.5°(c=2.00, H_2O). *Anal.* Calcd. for $C_{13}H_{16}O_6NNa$: N, 4.59. Found : N, 4.67.

Addition Product of Aniline N-Glucosyluronic Acid and Aniline—The compound is unstable, readily colors brown; m.p. 115°(decomp.). *Anal.* Calcd. for $C_{18}H_{22}O_6N_2$: C, 59.66; H, 6.12; N, 7.73. Found : C, 59.68; H, 6.08; N, 7.60.

Addition Product of *p*-Toluidine N-Glucosyluronic Acid and *p*-Toluidine—Obtained from *p*-toluidine (2 g.) and glucuronic acid (1 g.) as unstable crystals of m.p. 120°(decomp.). Yield, 0.8 g. *Anal.* Calcd. for $C_{20}H_{26}O_6N_2$: C, 61.52; H, 6.71; N, 7.18. Found : C, 61.48; H, 6.56; N 7.13.

Sodium *p*-Acetamidoaniline N-Glucosyluronate—Leaflets, m.p. 178°(decomp.). *Anal.* Calcd. for $C_{14}H_{17}O_7N_2Na$: N, 8.03. Found : N, 8.23.

Sodium *p*-Chloroaniline N-Glucosyluronate—Needles, m.p. 197~199°(decomp.). $[\alpha]_D^{23}$ −71.43° → −28.57°(c=1.40, H_2O). *Anal.* Calcd. for $C_{12}H_{13}O_6NClNa$: C, 44.25; H, 4.02; N, 4.30. Found : C, 44.30; H, 4.28; N, 4.59.

Sodium *p*-Dimethylaminoaniline N-Glucosyluronate—Colorless crystals, m.p. 225~228°(decomp.). $[\alpha]_D^{29}$ −8.71°→55.26°(c=1.14, H_2O). *Anal.* Calcd. for $C_{14}H_{19}O_6N_2Na$: N, 8.38. Found : N, 8.12.

Sodium *p*-Aminoazobenzene N-Glucosyluronate—m.p. 230~245°(decomp.). *Anal.* Calcd. for $C_{18}H_{18}O_6N_3Na$: N, 10.63. Found; N, 10.24.

***p*-Phenylenediamine N,N′-Diglucosyluronic Acid**—Obtained from a solution of *p*-phenylenediamine (1.6 g.) and sodium glucuronate (2.1 g.), adjusted to pH 5.5 with AcOH, as colorless needles, m.p. 192°(decomp.). *Anal.* Calcd. for $C_{18}H_{24}O_{12}N_2$: N, 6.09. Found. N, 6.30.

Ethyl Aniline N-Glucosyluronate—A mixture of aniline (4 cc.) and ethyl glucuronate (0.22 g.) was left at room temperature for 2 days. On addition of (*iso*-Pr)$_2$O, a crystalline product separated, which was recrystallized from (*iso*-Pr)$_2$O-EtOH. Yield, 0.15 g. m.p. 103~105°(decomp.). $[\alpha]_D^{17.5}$ +96.6°→ +36.6°(c=1.50, C_2H_5OH). *Anal.* Calcd. for $C_{14}H_{19}O_6N$: C, 56.56; H, 6.44; N, 4.71. Found : C, 56.25; H, 6.13; N, 4.85.

Ethyl Aniline (2,3,4-Tri-O-acetyl-N-glucosyl)uronate—Obtained by acetylation of ethylaniline N-glucosyluronate with Ac_2O and pyridine under cooling at 0°. Recrystallization from EtOH gaves needles, m.p. 160°. $[\alpha]_D^{13}$ +186.0°(c=1.715, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{25}O_9N$: C, 56.73; H, 5.95; N, 3.41. Found : C, 56.68; H, 5.77; N, 3.79.

Ethyl *p*-Aminoazobenzene N-Glucosyluronate—EtOH solution (25 cc.) of *p*-aminoazobenzene (3 g.) and ethyl glucuronate (3 g.) was heated for a while and left at room temperature for 2 days. An orange-colored precipitate was recrystallized from EtOH to orange-yellow needles, m.p. 133~134°. *Anal.* Calcd. for $C_{20}H_{23}O_6N_3 \cdot C_2H_5OH$: C, 59.05; H, 6.53; N, 9.39. Found : C, 59.01; H, 6.29; N, 9.46.

Tri-O-acetyl Derivate : Obtained as yellowish needles, m.p. 187°, by the same procedure as for acetylation. *Anal.* Calcd. for $C_{26}H_{29}O_9N_3$: C, 59.20; H, 5.50; N, 7.97. Found : C, 58.97; H, 5.18; N, 7.60.

Methyl [N-Methylaniline (2,3,4-Tri-O-acetyl-N-glucosyl)]uronate—A solution of N-methylaniline (2 g.) and methyl (2,3,4-tri-O-acetylglucosyl bromid)uronate (0.8 g.) in benzene (10 cc.) was stirred at room temperature after addition of Ag_2CO_3 (0.4 g.). Ag salt was discarded, the solution was evaporated *in vacuo*, and the residue was recrystallized from EtOH to fine needles, m.p. 149°. $[\alpha]_D^{25}$ +49.63°(c=2.013, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{23}O_9N$: C, 56.73; H, 5.95; N, 3.31. Found : C, 56.63; H, 5.96; N, 3.43.

Methyl [N-Ethylaniline (2,3,4-Tri-O-acetyl-N-glucosyl)]uronate—Obtained from N-ethylaniline as fine needles, m.p. 110°. $[\alpha]_D^{25}$ +57.5°(c=2.00, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{27}O_9N$: C, 57.66; H, 6.22; N, 3.20. Found: C, 57.67; H, 6.19; N, 3.37.

Methyl [*p*-Methylaminoazobenzene (2,3,4-Tri-O-acetyl-N-glucosyl)]uronate—Prepared from *p*-methylaminoazobenzene by the same procedure. The crude product was purified by chromatography over alumina and benzene as eluate. Recrystallization from EtOH gave yellow needles, m.p. 195.5~196.5°. *Anal.* Calcd. for $C_{26}H_{29}O_9N_3$: C, 59.19; H, 5.54; N, 7.97. Found: C, 59.27; H, 5.63; N, 8.00.

Thallium Aniline N-Glucosyluronate (II)—Obtained from Tl glucuronate and aniline by the above procedure. Recrystallization from its aqueous solution with acetone gave needles, m.p. 164~165°(decomp.). The same compound was also obtained from a mixture of sodium aniline N-glucosyluronate (I) and $TlNO_3$ in H_2O, and by subsequent precipitation with acetone. $[\alpha]_D^{19}$ −51.58°→−24.56°(c=3.59, H_2O). *Anal.* Calcd. for $C_{12}H_{14}O_6NTl$: C, 30.49; H, 2.98; N, 2.96. Found: C, 30.54; H, 2.97; N, 3.23.

The starting material, Tl glucuronate, was prepared from D-glucuronolactone and TlOH, followed by recrystallization from hydr. MeOH. The compound was also obtained from saturated solution of sodium glucuronate and $TlNO_3$ by precipitation with MeOH. Prisms, m.p. 146°(decomp.). $[\alpha]_D^{17}$ +9.11°→+12.64°(c=3.95, H_2O). *Anal.* Calcd. for $C_6H_9O_7Tl$: C, 18.13; H, 2.28. Found: C, 18.37; H, 2.21.

Methyl [Aniline (2,3,4-Tri-O-acetyl-N-glucopyranosyl)]uronate (IV)—i) To a solution of MeI (1 cc.) and dimethylformamide (2 cc.), 0.5 g. of (II) was added and the mixture was stirred at room temperature for 4 hr. Filtration of separated TlI and evaporation of the solvent gave a syrupy residue. The product was treated with Ac_2O (4 cc.) and pyridine (5 cc.), cooling with ice, and after standing overnight in an ice box, the solution was poured into cold water. The precipitate was recrystallized from EtOH to fine needles, m.p. 175~176°, $[\alpha]_D^{18}$ +143.86°(c=1.425, $CHCl_3$). *Anal.* Calcd. for $C_{19}H_{23}O_9N$: C, 55.74; H, 5.66; N, 3.42. Found: C, 55.87; H, 5.21; N, 3.37.

ii) Methyl (2,3,4-Tri-O-acetylglucopyranosylbromid)uronate (V) was prepared from methyl (1,2,3,4-tetraacetylglucopyranosyl)uronate and HBr by the usual way. The former material was obtained by acetylation of methyl glucuronate which was obtained from D-glucuronolactone and MeOH employing Amberlite IR-4B as a catalyst.

A mixture of 1 g. of (V) and 1 cc. of aniline was stirred and left overnight. The solution was poured into water and the precipitate was recrystallized from EtOH to needles (0.5 g.), m.p. 176°, $[\alpha]_D^{18}$ +144.00(c=1.375, $CHCl_3$). It did not show any m.p. depression on admixture with the substance obtained by the procedure (i).

Summary

In connection with the known fact that some aromatic amines are metabolized and excreted in urine as glucuronic acid conjugates, the N-glucosyluronates of primary aromatic amines, such as aniline, toluidine, *p*-chloroaniline, *p*-aminoazobenzene, *p*-dimethylaminoaniline, and *p*-phenylenediamine, were prepared. The same compound of secondary amines were not obtained. It was established that the conjugate should have a structure of N-glucopyranosyluronic acid, possibly in β-configuration, from the fact the acetylation product derived from methyl ester of glucuronic acid conjugate of aniline was identical with the compound prepared from methyl (2,3,4-tri-O-acetylglucopyranosylbromid)uronate and aniline.

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